Isolation rates of *Campylobacter fetus* subsp *venerealis* from bovine preputial samples via passive filtration on nonselective medium versus selective medium, with and without transport medium

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**Objective**—To compare the recovery rates of *Campylobacter fetus* subsp *venerealis* (Cfv) from preputial scrapings of infected bulls with passive filtration on selective medium versus nonselective medium, with and without transport medium.

**Samples**—217 preputial scrapings from 12 bulls (4 naturally and 8 artificially infected with Cfv).

**Procedures**—Preputial scrapings were collected in 2 mL of PBS solution and bacteriologically cultured directly on Skirrow medium or passively filtered through 0.65-µm filters onto blood agar, with or without 24 hour preincubation in modified Weybridge transport enrichment medium (TEM). After 72 hours, plates were examined for Cfv and bacterial and fungal contamination or overgrowth.

**Results**—Passive filtration of fresh preputial scrapings onto blood agar yielded significantly higher recovery rates of Cfv (86%) than direct plating on Skirrow medium (32%), whereas recovery from TEM was poor for both media (35% and 40%, respectively). Skirrow cultures without TEM were significantly more likely to have fungal contamination than were cultures performed with any other technique, and fungal contamination was virtually eliminated by passive filtration onto blood agar. Bacterial contamination by *Pseudomonas* spp was significantly more common with Skirrow medium versus passive filtration on blood agar, regardless of TEM use.

**Conclusions and Clinical Relevance**—The use of transport medium and the choice of culture medium had significant effects on Cfv recovery and culture contamination rates from clinical samples. Both factors should be considered when animals are tested for this pathogen. (Am J Vet Res 2013;74:1066–1069)

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>BA</td>
<td>5% sheep blood agar</td>
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<tr>
<td>Cfv</td>
<td><em>Campylobacter fetus</em> subsp <em>venerealis</em></td>
</tr>
<tr>
<td>TEM</td>
<td>Transport enrichment medium</td>
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</table>

The World Organisation for Animal Health lists Cfv as a notifiable disease and publishes guidelines for international testing and trade. Although Cfv-specific PCR assay protocols do exist, culture isolation using Skirrow medium is still the recommended diagnostic technique. This selective medium is blood based (containing 5% to 7% lysed, defibrinated blood) and contains the selective agents polymyxin B sulfate (2.5 U/mL), trimethoprim (5 µg/mL), vancomycin (10 µg/mL), and cycloheximide (50 µg/mL). As an alternative, a nonselective blood-based medium (5% to 7% blood) in combination with filtration (0.65-µm pore size) can be used, with the caveat that “it may be less sensitive when compared with a selective medium.” Filtration-based isolation methods for *Campylobacter* spp are variations of the original Cape Town Protocol, which has proven success in isolating many *Campylobacter* spp, including...
polyoxymycin B₉C [10 U/mL], cycloheximide [100 µg/mL], bacteriologic charcoal [0.5% (wt/vol)], base containing lysed horse blood (7% [wt/vol]) and human and animal feces to food and water.²⁻⁴

Transport medium is reported as essential for samples that cannot be processed in the laboratory within hours after collection.³ Several formulations of TEM have been proposed and tested for Cfv, with modified Weybridge TEM being the current superior choice.⁴⁻⁶ Modified Weybridge TEM is a Mueller-Hinton broth base containing lysed horse blood (7% [wt/vol]) and several selective agents.⁷ Given the different permutations recommended for Cfv isolation, the objective of the study reported here was to compare recovery rates of Cfv from infected bulls by use of Skirrow medium and passive filtration onto blood agar, with and without preincubation in modified Weybridge TEM.

Materials and Methods

Animals—The study was approved by the University of Saskatchewan’s Animal Research Ethics Board and adhered to the Canadian Council on Animal Care guidelines for humane animal use (protocol No. 2010077). The 12 bulls used in this study were housed together at the Western College of Veterinary Medicine at the University of Saskatchewan in a shared outdoor pen. Four bulls were purchased from local cattle operations according to established protocols.²⁰ Bulls were infected with Cfv isolated from 3 of the Cfv-positive bulls were confirmed Cfv-positive via bacteriologic culture.²¹ Four bulls were purchased from local cattle operations together at the Western College of Veterinary Medicine and transported to the laboratory for processing within 2 hours. The TEM samples were stored at room temperature (22° to 24°C) in the dark for 24 hours before being processed in the laboratory.

Bacteriologic culture—Fresh samples were thoroughly mixed, and preputial material was spread on Campylobacter agar Skirrow medium²² (300 µL of material) or BA plates²³ overlaid with a mixed cellulose ester membrane filter (0.65-µm pore size; 100 µL of material [BA100] or 300 µL of material [BA300]). Filter plates were incubated, filter side up, at 37°C for 30 minutes to allow motile cells to cross the membrane, after which time the filters were removed and all plates (Skirrow and BA) were returned to 37°C and incubated for 72 hours in a resealable pouch.¹ The TEM samples were bacteriologically cultured in an identical fashion, with 300 µL of enriched broth applied to both media types. Suspect Cfv colonies were identified macroscopically as small, smooth, translucent colonies and microscopically examined by use of Gram staining. Gram-negative cells with Campylobacter-like morphologies (rods or spirals) were confirmed as Cfv by use of a PCR assay protocol.⁶ Contaminating organisms were identified by sequencing of the cpn60 gene from representative colonies according to established protocols.²⁰

Statistical analysis—Generalized estimating equations with a logit link function and binomial distribution were used to compare the proportion of samples that were culture positive among different media types after accounting for natural versus artificial infection and repeated measurements in individual bulls. The same model was used to compare the proportion of samples that were contaminated with fungus or had overgrowth by Pseudomonas spp. Values of P < 0.05 were considered significant.

Results

Two hundred seventeen samples were evaluated in this study, with 114 samples taken from 4 naturally infected bulls and 103 samples taken from 8 artificially infected bulls (Table 1). There was no significant difference in the proportion of Cfv culture-positive samples between naturally and artificially infected bulls (P = 0.82). Passive filtration of fresh preputial scraping samples onto BA100 or BA300 resulted in significantly (P < 0.001) better recovery rates than all other culture methods examined, with 49 of 57 (86%) and 188 of 217 (87%) positive samples, respectively. Use of Skirrow medium with the same fresh samples yielded a recovery rate of only 66 of 208 (32%). After overnight incubation in modified Weybridge TEM, passive filtration onto BA and Skirrow medium yielded recovery rates of 43 of 124 (35%) and 50 of 124 (40%), respectively.

Table 1—Proportion (%) of positive bacteriologic culture results for Cfv in preputial scraping samples from 12 known infected bulls, obtained by use of 5 culture methods.

<table>
<thead>
<tr>
<th>Infection type*</th>
<th>No. of samplings</th>
<th>BA100</th>
<th>BA300</th>
<th>Skirrow</th>
<th>TEM BA</th>
<th>TEM Skirrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>114</td>
<td>30/36 (83)</td>
<td>103/114 (90)</td>
<td>32/108 (30)</td>
<td>27/62 (44)</td>
<td>22/62 (35)</td>
</tr>
<tr>
<td>Artificial</td>
<td>103</td>
<td>19/21 (90)</td>
<td>85/103 (83)</td>
<td>34/100 (34)</td>
<td>16/62 (26)</td>
<td>26/62 (45)</td>
</tr>
<tr>
<td>Both</td>
<td>217</td>
<td>48/57 (86)</td>
<td>68/217 (67)</td>
<td>66/208 (32)</td>
<td>43/124 (35)</td>
<td>50/124 (40)</td>
</tr>
</tbody>
</table>

*Four bulls were naturally infected, and 8 were artificially infected.

BA100 = Passive filtration onto 5% sheep BA of a 100-µL sample. BA300 = Passive filtration onto 5% sheep BA of a 300-µL sample.
Even in those small studies, all authors reported contamination issues that inhibited Cfv isolation from at least 1 bull. The most common contaminants reported include *Pseudomonas* spp, *Proteus* spp, and fungi.4,10,22

In the present study, *P. aeruginosa* and fungi were regularly detected. *Pseudomonas aeruginosa* was cultured from half the bulls in the present study (6/12), with virtually all contamination occurring on Skirrow medium. Preincubation of preputial scraping samples in TEM had no effect on *P. aeruginosa* contamination. The similar performance of modified Weybridge TEM and Skirrow medium was not unexpected because Weybridge TEM contains the same selective agents as Skirrow medium.9,10 The presence of *P. aeruginosa* was important because the organisms typically covered the entire surface of the culture plate, preventing recovery of other bacteria. Conversely, samples from all 12 bulls had fungal contamination on Skirrow medium alone but little contamination on blood agar after filtration or with preincubation in TEM. This suggests that the physical barrier of the filter or the combined effect of cycloheximide from the TEM and Skirrow medium (100 µg/mL and 50 µg/mL, respectively) is a more effective antifungal agent than the cycloheximide concentration in Skirrow medium alone.

Samples preincubated in modified Weybridge TEM did not yield a significant difference in Cfv recovery rates between culture media, reducing successful culture rates via both media types to only 35% to 40%. Therefore, passive filtration of fresh preputial samples on blood agar was the superior isolation method for Cfv. When this option is not available (for example, because of prolonged sample transport time), laboratories should anticipate a high false-negative rate for Cfv culture.

**Discussion**

In this study, bacteriologic culture of fresh preputial scraping material via passive filtration on BA recovered Cfv from 86% to 87% of known positive samples. Bacteriologic culture of the same fresh preputial scraping samples on Skirrow medium resulted in a mean Cfv recovery rate of only 32%. Previous attempts have been made to examine Cfv culture recovery rates from known infected bulls, with study cohorts of 4 bulls (78 samples),21 5 bulls (100 samples),4 and 1 bull (9 samples).3 Even in those small studies, all authors reported contamination issues that inhibited Cfv isolation from at least 1 bull. The most common contaminants reported include *Pseudomonas* spp, *Proteus* spp, and fungi.4,10,22

In this study, bacteriologic culture of fresh preputial scraping material via passive filtration on BA recovered Cfv from 86% to 87% of known positive samples. Bacteriologic culture of the same fresh preputial scraping samples on Skirrow medium resulted in a mean Cfv recovery rate of only 32%. Previous attempts have been made to examine Cfv culture recovery rates from known infected bulls, with study cohorts of 4 bulls (78 samples),21 5 bulls (100 samples),4 and 1 bull (9 samples).3 Even in those small studies, all authors reported contamination issues that inhibited Cfv isolation from at least 1 bull. The most common contaminants reported include *Pseudomonas* spp, *Proteus* spp, and fungi.4,10,22

The most common bacterial contaminant detected in this study was determined to be *Pseudomonas aeruginosa* on the basis of its gray-green colony morphology on Skirrow medium, appearance as a gram-negative rod, and having a cpn60 sequence 99% identical to reference *P. aeruginosa* sequences. The occurrence of *P. aeruginosa* contamination was significantly (P < 0.001) more frequent when Skirrow medium was used with or without preincubation with TEM than when BA300 was used (Table 2). The 2 bulls with the highest rates of *P. aeruginosa* contamination on Skirrow medium (65% to 80% of plates contaminated) correspondingly had the lowest recovery rates of Cfv from that medium (0% to 13%). In contrast, the same 2 bulls had no *P. aeruginosa* contamination on BA with passive filtration, leading to much higher Cfv recovery rates (78% to 88%).

The other major contaminant encountered was fungus. The prevalence of fungal contamination was significantly (P < 0.001) higher with Skirrow medium than with any of the other culture methods examined. The prevalence of fungal contamination among bulls was quite variable when culturing fresh preputial material: 1 bull had only 7% (2/30) of Skirrow plates contaminated, whereas another bull had 9 of 9 plates contaminated. Overall, for fresh preputial scraping samples, 5 of 208 (2%) BA300 plates and 77 of 183 (42%) Skirrow plates were contaminated with fungus (Table 2). The surface area of fungal growth on the plates ranged from < 20% to 100%. The 4 bulls with > 50% of their fresh cultures contaminated with fungus (73%, 67%, 100%, and 86%) had correspondingly low Cfv recovery on Skirrow medium (53%, 38%, 11%, and 29%, respectively). As with *P. aeruginosa*, passive filtration virtually eliminated fungal contamination. Conversely, culturing after preincubation in TEM virtually eliminated fungal contamination on either medium, with only 2 bulls having fungal contamination on TEM Skirrow medium (2/21 plates and 2/17 plates).

**References**

2. World Organisation for Animal Health. Bovine genital campylo-

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Table 2—Proportion (%) of Cfv bacteriologic culture plates of preputial scraping samples from 12 known infected bulls contaminated with either fungus or *Pseudomonas aeruginosa*.

<table>
<thead>
<tr>
<th>Infection type*</th>
<th>BA300</th>
<th>Skirrow</th>
<th>TEM BA300</th>
<th>TEM Skirrow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Natural</td>
<td>5/108 (5)</td>
<td>36/93 (39)</td>
<td>0/62 (0)</td>
<td>2/62 (3)</td>
</tr>
<tr>
<td>Artificial</td>
<td>0/100 (0)</td>
<td>41/90 (46)</td>
<td>0/62 (0)</td>
<td>2/62 (3)</td>
</tr>
<tr>
<td>Both</td>
<td>5/208 (2)</td>
<td>77/183 (42)</td>
<td>0/124 (0)</td>
<td>4/124 (3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungus</th>
<th>BA300</th>
<th>Skirrow</th>
<th>TEM BA300</th>
<th>TEM Skirrow</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>1/112 (1)</td>
<td>34/108 (31)</td>
<td>1/82 (1)</td>
<td>27/82 (44)</td>
</tr>
<tr>
<td></td>
<td>0/103 (0)</td>
<td>22/100 (22)</td>
<td>0/82 (0)</td>
<td>17/62 (27)</td>
</tr>
<tr>
<td></td>
<td>1/215 (0)</td>
<td>57/208 (27)</td>
<td>1/124 (1)</td>
<td>44/124 (35)</td>
</tr>
</tbody>
</table>

See Table 1 for key.


10. Le Roux E, Lastovica AJ. The Cape Town Protocol: how to isolate the most campylobacters for your dollar, pound, franc, yen, etc. in Proceedings. 9th Int Workshop Campylobacter Helicobacter Relat Organ 1998;30:30–33.


